Newsletter for June 2024

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Engineering the elastic properties of scales

By Lathreas and Tiltwolf | June 30, 2024

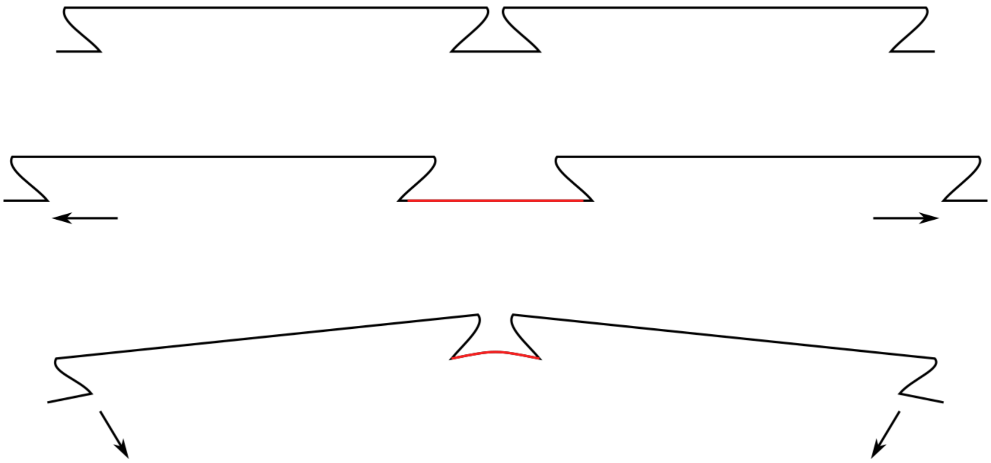
Engineering complex systems is a difficult task. For that reason, the first steps of engineering usually entail listing what it is that we actually want to achieve. The shorter the list, the easier the task becomes. In terms of the integument (fur/feathers/scales), we care about the functional properties of the integument — these are the things that make the integument look, feel and behave in the way we expect. In a previous newsletter article, we have extensively talked about the functional properties of fur, and strategies on how to recreate those properties in humans. We can do the same for scales. If all relevant properties are recreated, we will have successfully reconstructed scales in humans.

Scales in nature have a huge diversity in both form and function. Most of them provide protection, but the size of scales, the organization, and even the way they are formed differs drastically. Many species have unique evolutionary adaptations, such as the smooth, ‘tiled’ arrangement of scales on various snake species giving rise to excellent slithering performance, the small, bump-like arrangement on iguana skin giving rise to better flexibility of the skin, and the strong and large scales of a pangolin giving the creature a tough hide.

One crucial component of scales is their elastic properties. Scales are different from fur and feathers in that they are flat plates, rather than upright protruding structures. Their flexibility, stretchiness, and shape strongly affect the ability of skin to bend. This is especially important in regions of the skin where bending can occur in many directions, such as near the armpits, neck, elbows, and so forth.

The effect that scales have on skin bending is mostly determined by the elasticity of the two main components that make up a scale. The hard, plate-like part of a scale is called the *scale plate*, and the soft, stretchy skin linking neighboring scales together is called a *scale hinge*. In snakes, the scale hinge is incredibly flexible and stretchy, to make up for the relatively rigid scale plates.

Figure 1: the scale plates (black outlines) are less stretchy than the scale hinge (red outline). This causes most of the deformation to be concentrated on the very flexible hinge region.



Not only do these properties — flexibility, stretchiness, hardness, and so forth — affect how the skin bends, but they also affect the aesthetics of the scaly skin in profound ways. Touching scales should feel like the real deal, and not like brittle and crusty skin, scar tissue, or a mere subdermal swelling. This requires correct structuring of the layers that make up a scale, correct application of the pigment layers, and so forth. Furthermore, all of these considerations require the ability to properly tune the scale's *material properties*. Several material properties are important, among which the Young's modulus, flexural modulus, surface roughness, hardness (both scratch resistance and deformation resistance), and friction coefficient. But how can we alter these properties?

The elastic properties of scales are determined by their chemical makeup. Most importantly, these properties are determined by structural biopolymers that keep the tissue bound together, such as keratin, collagen, and elastin. These classes of proteins are present in both reptiles and humans, but differences in their structural arrangement and concentration change how they affect the material properties of the integument.

In virtually all mammalian species, deformation hardness — an important material property — is regulated by alpha-keratins. They are loosely divided into "hard" and "soft" keratins, and they form long interconnected fibrils of varying rigidity. Hardness is partially controlled by the degree of crosslinking, i.e. how many bridges exist between each alpha-keratin fibril and its neighboring fibrils. These are called disulfide bridges, and they rely on the presence of cysteine, an amino acid, in the keratin sequences. Indeed, "hard" keratins have a much higher cysteine content, and are thus able to make many more bridges, rendering their networks much more rigid and hard.

Hard keratins are mostly expressed in hair and nails, whereas soft keratins with lower cysteine content are found more in the surrounding skin. It is at least theoretically possible to induce hard keratins to be expressed in skin, which is likely to result in substantial hardening. However, this doesn't represent a complete picture, because although the skin will be harder, it may also be more brittle. Additionally, it would be very important to have borders between scales where these hard keratins are not expressed, allowing for sufficient flexibility.

In reptiles and avians, there are also beta-keratins, which contribute much of the hardness found in scales and feathers. (*Editor's note: beta-keratins are more properly referred to as "corneous beta proteins" or "keratin associated beta proteins", but "beta-keratin" terminology has a longer history in the field. All these terms relate to the same family of proteins.*)

Historically, it has generally been believed that beta-keratins act as crosslinkers to render alpha-keratin fibrils as more rigid, by forming disulfide bridges with multiple alpha-keratin filaments at once. However, it was somewhat more recently shown that in feather barbules, the vast majority of the keratinous mass comes from beta-keratins alone, which form a rigid matrix on their own by polymerizing with each other (Alibardi, 2013). They showed this by a procedure called immunogold labeling, where gold nanoparticles are coated in antibodies against alpha- and/or beta-keratins and then exposed to feather tissue, which can then be imaged by electron microscopy. The gold particles appear as black dots on these images and can tell us how much of these proteins are present in different parts of the tissue.

These beta-keratin-dominant tissues probably have significantly different material properties, and to our current knowledge, it isn't known whether this beta-keratin dominance also exists in scales. An interesting research question would indeed be to repeat the immunogold labeling experiment with scale tissue to determine whether the same beta-keratin dominance exists in this situation.

An important open question for our purposes is whether beta-keratins are necessary for generating sufficiently hard scales that are still strong enough to withstand everyday use, i.e. that are not too brittle. It's possible to test this either *in vitro* or *in vivo*. In the latter case, one may be able to knock out the beta-keratin gene cassette from a reptilian genome to then observe the differences in adult scale properties. However, we would prefer to avoid animal research wherever possible, and thus it may also be interesting to attempt to recreate existing methods for reconstituting human skin *in vitro* from stem cells, but using cells cultured from reptiles instead. If this turns out to be feasible, it would become possible to mutate and test the effects of various keratins and other structural proteins on the material properties of the scales grown *in vitro*.

An alternative approach would be to start with human skin grown *in vitro*, which is well-described in the literature, followed by inserting copies of hard keratins and/or beta-keratins to observe the change to material properties. If we first obtain a baseline rheological profile of human skin and reptilian scales by means of biophysical assays, we can then compare the effect of adding different combinations of these structural proteins to human skin in an attempt to nudge it towards a reptilian phenotype.

Luckily, although useful information, we do not need to fully understand the exact chemical details of reptilian scales to be able to reconstruct their properties in human skin. Again, simply copying nonhuman genes into humans can lead to all kinds of nasty side-effects, such as immune rejection. It is far more important to understand how human skin works, as that is the system we seek to alter. Knowing that the level of hardness is mainly determined by the degree of crosslinking in keratins or other structural proteins, a feasible strategy could be to simply alter the tissue composition using slightly altered pathways and proteins that are already present in humans.

There are many different human keratins, only some of which are expressed in skin. All human keratin types combined, however, show a wide range of different rheological properties, which depends only on the exact mixture of biopolymers used and their degree of crosslinking. For example, the keratinous material that makes up your fingernails shows excellent hardness, showing that purely human hard plates are certainly possible. Although this is not the exact type of material that we would seek to construct as a substitute for scales, carefully tuning the mixture of keratins (e.g. by altering their expression in skin) is expected to give rise to a wide palette of different material properties, from soft and flexible to hard and strong, including one exactly matching the natural scale type of the patient's choice. The upside of this approach is that none of the materials used would be foreign to the body, meaning that there is little chance of immune rejection. But of course, orchestrating human skin to behave in a different way requires a thorough understanding of the biochemistry and biophysics of skin tissue.

Regardless of the approach, we will require a good characterization of the elastic properties of natural scales, such that we have proper targets to engineer for. Several simple experiments exist that can give us this data, such as one in which we carefully press on a scale sample with a well-calibrated force-generating device to measure the stress-strain curve of the material.

There are also still many fundamental questions waiting to be answered, such as how keratinization is regulated in different tissues, how tissues transport and orchestrate the arrangement of structural proteins, the various effects that the chemical environment can have on folding and network arrangement, the effects of cell (de)hydration, and much more. But that's also a fun part of science! We are currently brainstorming through which experiments are the most important to do first, to elucidate the knobs we can turn to properly control the hardness and elasticity of skin, which may be relevant even for patients desiring something like cetacean skin or insectoid plates. Of course, work on fur and feathers is also continuing, with some interesting research in the proposal pipeline that we will comment on in a later newsletter.

We are currently hard at work wrapping up the final pieces for chapter 3 (Properties of Scales) of the integument review project, with just a few more things to check, and are also well on our way with chapters 5 and 7 about the developmental biology of human skin and feathers. Stay tuned for more!

More details about our Integument Review project are at *https://freedomofform.org/research/project-integument-review/*

Volunteer Spotlight: Tiltwolf

By SvarOS | June 29, 2024

An interview with our Chief Science Officer

Introduction:
Tilt is a Canadian biochemist, bioinformatician, and transhumanist. Their journey includes collaborative biochemistry, cell biology, and bioinformatics research for their PhD in Ontario, Canada. They also studied biomedicine, chemical biology, and computational neuroscience during their previous undergrad degree. Their achievements also include serving as an External Collaborator of the Blue Brain Project at the Swiss Federal Institute of Technology in Lausanne [École Polytechnique Fédérale de Lausanne, or EPFL], and on the Freedom of Form Foundation (FoFF) side, conducting the first preliminary experiments demonstrating cosmetically modifying cells to control hair and skin cell behavior. As a researcher, their focus is mechanistic biology and protein engineering. With the support of the FoFF, they have established a small wet lab space dedicated to FoFF-related research. Their current focus is on manipulating the behavior of skin and hair to become more animalistic in look. Their passion for their work is palpable, and it is this passion that drives their research forward. Their hobbies include philosophy, playing their antique Heintzman piano, and creating floral ceramic art in their spare time.

Interview:
SvarOS: What's your role in the organization for those unfamiliar with you?

Tilt: As one of the foundation's lead scientists, I currently hold the positions of Vice President and Chief Science Officer (CSO). My primary focus is on the biological side of research in morphological freedom, a role I undertake with a deep sense of responsibility and dedication.

SvarOS: Where did you hear about the Foundation, and when did you join?

Tilt: I learned about the FoFF in August 2020 and was immediately very strongly drawn to its mission. Upon reaching out, I had the pleasure of meeting Zennith and Syralth to discuss the science of freedom of form. Our shared values and vision for the future led to my officially joining the server in September 2020. I later volunteered in early 2021, when I initiated the integument [skin] research project. This journey to joining the FoFF reflects my deep enthusiasm and alignment with the Foundation's mission.

SvarOS: I understand that the integument project involves a lot of genetic and biological wizardry. Could you explain to the reader what genetic manipulation is?

Tilt: One of the significant challenges to achieving nonhuman characteristics is changing the entire skin surface to match the destination species. Many of the necessary body changes can be achieved with surgery, but the skin is different, and there are no apparent methods for inducing widespread fur or scale growth. To do this, we need to activate cell behaviors that are usually only active during early development - processes that they can do, but that they turn off when we become adults. So, it's not just messing with genetics; you need a combination of genetic engineering and exposure to signaling molecules that aid in cellular reprogramming.

Let's say we want transplantable follicles, to give a practical example, for fur growth. You can take cells from a patient and start growing them in the lab, then transform them into stem cells that we can reprogram into other tissue types. The methods for making stem cells are mainly based on inserting extra copies of three to four key genes, called Yamanaka factors, into the patient's cells. Once you have the stem cells, you can expose them to different specific morphogens. Morphogens are proteins that change how your cells organize themselves - to reshape them into functional hair follicles.

Part of my role has thus been to disentangle the biological pathways that control fur, scales, and feathers to recreate them in human skin in adults, and that brings together a combination of gene research, stem cell biology, and developmental genetics. In our research within the integument team and our newly forming wet lab projects, we're studying biological pathways that we can use to fine-tune these follicles in vitro before implanting them. Different destination species require different kinds of hairs with unique textures and colors - it isn't enough to load the skin with a ton of regular human hair to get a realistic result. So, what we're doing is studying the pathways that control essential parameters - knobs we need to be able to turn for this purpose. Those knobs include things like hair thickness, length, coloration, density, and so on, which are each controlled by specific morphogens. For example, we've found that you can control hair thickness and length by tuning the relevant genetic pathways (specifically Wnt10b and FGF5, respectively).

SvarOS: I also understand your team identified a recent discovery that makes it possible to generate “hairballs.”

Tilt: In a way, yes! During our deep dive into the integument review into practical laboratory methods, we found a study by Dr. Karl Koehler’s group at Harvard that showed a complete working method for making these hair-bearing organoids. They’re like little inverted balls of skin cells, with miniature hairs growing inwards towards the middle. Indeed, you can most likely use them to “plant” new hairs in an adult human.

What was particularly striking about these “hair-bearing organoids” is how they were made. We’ve previously identified many important pathways for early hair follicle development, especially specific protein Wnt and BMP signaling. Unexpectedly, it turns out that you don’t need to poke at Wnt signaling at all to make these things! Indeed, all you need is two cocktails applied to the cells in order: one with high BMP4, low FGF, and a TGFβ inhibitor, and a second one with a BMP inhibitor and high FGF. Strikingly, that’s enough to start a chain reaction that makes the other pathways correctly activate, leading to the cells fully reorganizing into viable hair follicles.

The cool thing is that if you insert intact “hairballs” - I love that term, I’m going to start using it - into very small 1mm (0.04 in) incisions into the skin, they readily turn themselves inside out and heal in place, leaving new hair follicles that point themselves towards the skin surface all on their own. Our task is to be then able to take these things and “tune” them to match destination species characteristics in a highly customizable and patient-centric way. It’s quite exciting!

SvarOS: Going beyond the Foundation for a moment, what exciting breakthrough in this field have you personally contributed to and can share?

Tilt: Regarding my research, I study short linear motifs in proteins that facilitate protein-protein interactions inside cells, which are critical for intracellular signaling. I'm interested in being able to model protein sequence motifs in "intrinsically disordered regions" (IDRs). To take a step back, you can think of a folded protein as a semi-rigid structure with different modules and flexible linkers (IDRs) between them. We used to think that IDRs weren't crucial for much except connecting the modules, but this turned out to be wrong - indeed, a very large number of critical protein interactions are mediated by them. Predicting sequence motifs in an IDR that will interact with another protein has historically been problematic because, unlike other parts of rigid protein structures, it's difficult to predict how sequence differences affect their behavior.

SvarOS: That sounds pretty critical. What methods have you developed or customized to fit your research to help you investigate these more deeply?

Tilt: The traditional way to deal with this is to take all of the protein sequences that you know can bind to your protein receptor of interest and stack them up to get a statistical distribution, but this gets messy for long variable motifs. You can also apply neural networks, but it requires huge amounts of data, and it's also a "black box" that doesn't tell you anything about why one sequence interacts while another similar one doesn't. I needed to figure out how to robustly predict these interactions in a way that would be highly interpretable and accurate.

I've been excited to discover during my PhD that there is a way to do this with non-neural machine learning - a method I developed that I've been calling "context-aware weighted matrices." Basically, you start with a purified receptor protein and an extensive array of synthetic possible motifs that could interact with it. Then you measure how strong each motif binds and feed that into the model during training. It will then look for contextual relationships in the sequences to determine which "real" motifs can interact with the receptor, and it represents these in a series of matrices that are highly interpretable. As it turns out, this works really well, with an accuracy of about 92% on our test data for our protein/motif combination of interest.

Better still, we can say **why** the model makes any given prediction, allowing us to go back and test if the model's inferences are right - which, in our test cases, they were! It's very cool and satisfying to work on these models, make some complicated predictions, and find out that indeed you were right - really exciting to experience.

SvarOS: Your interest in bioinformatics goes back quite a bit. You mentioned working for the Blue Brain Project at EPFL. What is the Blue Brain Project, and what did you do while participating in it?

Tilt: The Blue Brain Project is a large-scale computational neuroscience project that has aimed to simulate the brain on very large IBM Blue Gene/Q supercomputers for the last decade or so. When I was working on it, we were particularly interested in jumping from simulated neural networks - which treat neurons like abstract math functions - to simulating the dynamics **inside** of neurons. So, for my senior undergraduate thesis, my part of this was to write code to simulate the calmodulin pathway, which controls how calcium affects electrical activity in neurons. It turned into quite an exciting project that was continued further by a postdoc after I left to do my PhD. It was my first foray into bioinformatics and simulated biochemistry and was a very formative experience in my research career.

SvarOS: So, what was the idea behind this? To simulate diseases or to learn more about cells?

Tilt: These simulations aim to understand the inner workings and emergent phenomena contributing to neural networks, ultimately controlling our brains' functions. The long-term goal is to simulate internal neuronal biochemistry with high enough fidelity that we can use it to test targets for neurological illnesses. Imagine if you could model Alzheimer's disease in a computer and then throw thousands upon thousands of simulated drugs at it to see which could restore healthy function - it would be huge for biomedical research in these areas.

To get a bit technical and loop it back to what I answered earlier, calcium is involved in a process called glutamatergic signaling (among MANY others), which is considered essential for many higher-level cognitive functions. My role was to demystify what happens when calcium enters a neuron during that signaling.

SvarOS: You seem to have quite a finger on the pulse of this intersection of biology and computing. With your insight, what limitations does this technology have, and what challenges do you feel are the most important to overcome?

Tilt: The body is a wild mess of complicated interactions at many layers of organization, and nudging a new system into just the right shape to make it work seamlessly is a big part of our work.

On the cell biology side, one of our main limitations is that the usual methods for creating stem cells carry some risks that make them less than ideal for transplanting into humans. These risks mainly come from needing to insert extra copies of stemness-related genes that stay in those cells forever, leading to the risk that stem cell-derived cells may de-differentiate and create complications.

Significant limitations exist for the more exotic integument types, like scales and feathers, as well. They require many additional nonhuman genes to function correctly in human cells, which will require much in vitro experimentation in the coming years.

Fortunately, recent research seems to indicate these problems are very solvable. For the extra copies of genes we're inserting, several recent methods have been described for making stem cells by only transiently introducing those stemness genes so that they disappear afterward and render the cells normal. One of the things I'm very interested in testing is trying to derive patient stem cells with those newer, safer methods and then applying the hair organoid procedure to see if we can still produce viable follicles. It's very likely to work, although it may require a lot of tinkering and optimization - but that's what science is all about. Regardless of the outcome, safety is paramount for what we're doing, especially when editing many genes involved in cell growth and function.

SvarOS: How do you think the Freedom of Form Foundation fits into the broader scheme of transhumanism?

Tilt: Transhumanism is the philosophy that we can and should use technology, including biotechnology, to change and improve our bodies to better fit who we are as individuals. I think it was most eloquently described by Dr. Max More in the 1990s in the idea of 'morphological freedom,' which proposes that you should not be captive to your biology but instead be empowered to change it for the better. For me, the Freedom of Form Foundation is the real-life manifestation of that philosophy. We are here to do the legwork and create/enable the technologies required to make these choices possible.

SvarOS: What do you think the organization's greatest challenge will be? Will it come from inside or outside the organization?

Tilt: I think it'll be some combination of both. On the inside, we will certainly encounter various scientific challenges that we'll have to engineer our way around, which is just part of the scientific process. On the outside, there will probably be some societal resistance to overcome, as always comes with any organization backing a paradigm-shifting philosophy.

More generally, though, I suspect that the biggest challenge will be convincing the wider community to invest on a large scale in our work. We've been lucky to have an incredible grassroots support base to enable our work.

The task now is to keep the momentum going to get a snowball effect happening. As we make more discoveries and prove ourselves by showing significant scientific progress in our goals, we will gain more and more attention from interested parties that want to see us succeed, hopefully translating into the funding we need to make it happen. Biomedical experimentation is notoriously expensive, but we're on track to get where we need to be.

SvarOS: What modification do you look forward to the most?

Tilt: Hmm, good question! In the near term, I would love to have canine claws. But as more becomes possible, I think having a proper muzzle and matching ears will be the most important thing to me. I dream of a moment when I can look into a mirror to see the real me smiling back.

SvarOS: So... Tell me about the piano. What joy does it bring to your life? What songs do you like to play?

Tilt: For me, music brings a lot of joy, especially songs with powerful meanings and feelings driving them. When I play a piece on the piano, I can fully immerse myself in the song and genuinely relax. I play a variety of kinds of music, but some of my favorites would have to be “How to Save a Life” by The Fray and “Bring Me to Life” by Evanescence. These are songs I find have a genuine heart to them.

SvarOS: Do you have a place to look for ceramics? What are your favorite designs? Who influenced you in your designs?

Tilt: I've been working with a local ceramics instructor specializing in 'experimental ceramics'—interpretive pieces with symbolic meanings and novel processes. His work combines Western and Eastern traditions, especially those from Japan, where he originally mastered this medium.

One of the little philosophical thoughts I like from his school of thought is that ceramics should be considered impermanent and meant to fulfill a purpose. So, if you make something unique and use it often, and one day it breaks, this is not a reason to avoid using your fancy pieces. Instead, it's a realization that the piece has fulfilled its purpose throughout its life in being used as intended.

I mostly experiment with larger bowls and vases (think one to two feet [30 to 60cm] wide). I then do intricate painting in underglaze, followed by novel glazes that give interesting textures and iridescent effects. At the moment, I'm working on a 12-inch [30 cm]-tall bowl with the silhouette of ivy vines woven around the edges.

SvarOS: Do you have any parting advice for those who want to join us or are curious about what we're doing or planning?

Tilt: I would say that for everyone interested in joining us, don't be afraid to speak up and ask how to dive into our research with your unique skills. The task of freedom of form is very multidisciplinary, and if you have an interest or passion in a particular area of this kind of research, chat with one of our scientists. We're all friendly and will be excited to help you find a place to chip in!

For folks curious about what we're doing and planning, I would also say that you should feel free to engage with us with your questions and thoughts and follow our public progress. We're a community-driven organization, so we are always interested in engaging with the community's questions and feedback.